**Phenotypic Characterization of Vaginal Candidiasis in Sudanese Pregnant Women**

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**Abstract**

**Background:** Pregnant women presenting with vaginal symptoms often complain of an abnormal discharge and possibly of other symptoms such as offensive odor or itching. *Candida* species are well recognized causes of vaginitis. Vaginal candidiasis is common and many non-specific infections may occur as a result of vaginal resistance leading to vaginal epithelial atrophy.

**Objective:** To assess the phenotypic characterization of vaginal candidiasis in Sudanese pregnant women using conventional laboratory techniques.

**Materials and methods:** 288 pregnant women aged 15-49 years were randomly investigated during the period from January 2008 to December 2010. Two high vaginal swabs (HVS) were collected from each patient and examined by microscopical wet-mounts and Gram stain. They were also cultured on Sabouraud dextrose agar (SDA), chrom agar medium, and corn-meal agar medium. *Candida albicans* was identified by colonial morphology, germ tube test, and sugar utilization tests. Non-albicans *Candida* species were differentiated by colonial morphology on chrom agar medium. Antifungal sensitivity tests were done by the cup-plate agar diffusion method. Antifungal drugs tested included clotrimazole, fluconazole, itraconazole, and nystatin.

**Results:** The study revealed that the frequency rate of vaginal candidiasis was 32.6%. The most predominant *Candida* species isolated was *Candida albicans* (81%) followed by *C. glabrata* (7.4%), *C. parapsilosis* (6.5%) and *C. tropicalis* (4.6%). 48.1% of samples were positive by Gram stain, 86.5% were able to grow on chrom agar medium, and 62.5% were able to grow on Sabouraud dextrose agar. *Candida albicans* was found more sensitive (69.8%) to clotrimazole than nystatin (8.7%) antifungals. Vaginal candidiasis was found more prevalent in the age range 20-29 years, and during the third trimester pregnancy.

**Conclusion:** The frequency rate of vaginal candidiasis was 32.6% among pregnant women, and *Candida albicans* was the commonest species isolated. The most effective antifungal drug for treatment of candidiasis was clotrimazole which was found superior to nystatin.

**Key words:** Vaginal candidiasis, Pregnant Sudanese women, Phenotypic characterization.
Introduction

Inflammation of vaginal mucosa (vaginitis) is a common clinical syndrome which appears to be increasing. Specific infections, including those transmitted sexually may be due to organisms such as *Neisseria gonorrhoeae*, *Mycoplasma*, and *Chlamydia*. Candida infection is perpetrated by the high glycogen content of vaginal epithelium. The lining of the normal female genital tract is a mucosal layer made up of transitional, columnar and squamous epithelial cells.

Various species of commensal bacteria colonize these surfaces, causing no harm to the host except under abnormal circumstances; and helping prevention of adherence of pathogenic organisms. The number of the female genital tract flora varies with pH and estrogen concentration of the vaginal mucosa, and with women age. Women in the reproductive age may harbor large number of facultative bacteria such as *Enterobacteriaceae, Clostridia*, streptococci, and staphylococci; as well as lactobacilli and anaerobic non-sporing bacilli and cocci.

Lactobacilli are the predominant organisms in vaginal secretions of normal healthy females. Recent studies had shown that hydrogen peroxide-producing lactobacilli are mostly associated with a healthy state. Many women carry *Streptococcus agalactiae* which may be transmitted to neonates. Although yeasts that are acquired from the gastrointestinal tract may be transiently recovered from the female vaginal tract, they are not considered normal flora.

Female genital tract infections are located anatomically either in the lower genital tract (vulva, and vagina) or in the upper genital tract (uterus, fallopian tubes, and ovaries). Although organisms causing lower tract infections are not usually part of the normal genital tract flora, some organisms that are normally present in very few numbers can increase sufficiently to an extent to cause infection. There seems to be a conspicuous absence of local or national on-going programs directed specifically towards vaginal infection during pregnancy. It is therefore hoped that this study will offer a hand in clarifying the laboratory diagnosis of vaginal candidiasis in pregnancy for integration in health care in Sudan. The available published literature on occurrence of candidiasis in Sudan is scarce. Reports on this subject were published by Omer (1977) and Ortishi, *et al* (2004). Vaginal candidiasis is the most common cause of vaginitis in Europe and Africa. It is the second common cause of vaginitis in United States. In general practice the diagnosis of vaginal candidiasis is based on clinical symptoms, and laboratory confirmation is rarely requested by clinicians.

Vaginal candidiasis is routinely diagnosed sometimes even without the use of microscopy. *In vitro* cultivation and serology are not usually adopted in Sudan due to practical difficulties and high cost. Hence about half the cases of candidiasis are misdiagnosed. The object of the present study was to perform microscopical, cultural and susceptibility techniques to diagnose vaginal candidiasis.
Materials and methods

This is a cross-sectional study, performed to isolate and identify Candida species causing vaginal candidiasis in Sudanese pregnant women. Patients included in this study were recruited from the antenatal clinic of Omdurman Maternity Hospital (Sudan) between January 2008 and December 2010. Inclusion criteria were pregnancy, presence of vaginal discharge, and 15-49 years age range. All patients were examined by the clinic obstetricians. A structural questionnaire was conducted to collect demographical and clinical information such as age, education level, occupation, occupation, parity, pregnancy trimester, history of vaginitis, vaginal discharge and clinical picture. The sample size investigated (288 patients) was calculated according to the equation:

$$\text{Sample Size} = \left(\frac{Z}{2}\right)^2 \frac{PQ}{d^2}$$

Where: $Z$ = Standard normal deviation which corresponds to 95% confidence; $P$ = Expected prevalence; $Q = 100 – P$; $d =$ Level of accuracy desired (standard value of 0.05).

Ethical approval of the study was obtained from the Research Board of the Faculty of Medical Laboratory Science, Omdurman Islamic University and the Research Committee of the Sudan Ministry of Health. Verbal consent was obtained from all participant patients.

Two high vaginal swabs (HVS) were collected from each patient using sterile, disposable vaginal specula. The first swab was used for preparing wet-mount microscopical examination. The second swab was inoculated in Amies transport medium for further cultural examination. All swabs were fully labeled and subjected for laboratory investigations.

A high vaginal swab (HVS) was mixed with normal saline solution on a glass slide and examined under the microscope for hyphae or budding yeast cells. Swabs were also examined by Gram stain to detect Gram positive budding yeast cells. HVS were then inoculated on Sabouraud dextrose agar (Oxoid) and incubated at 37 °C for 48 hours. Plates were examined for growth of Candida. This growth was characterized by a distinct yeast smell and presence of budding on viewing under the light microscope. Candida albicans forms smooth, creamy colonies with a moderate size on Sabouraud dextrose agar. Candida albicans was further confirmed by germ tube test and chlamydomospores production on corn meal agar. Culture on chrom agar medium (Conda Limited, England) was performed to differentiate between Candida species morphology as follows: Candida albicans (light green, smooth colonies), and Candida glabrata (creamy-white smooth colonies), Candida tropicalis (blue-metallic, raised colonies), and Candida krusi (pink, fuzzy colonies). Sugar fermentation (zymogram) and sugar assimilation (auxanogram) of glucose, lactose, maltose, sucrose, were also performed to differentiate between Candida species. The fungal growth was harvested from Sabouraud dextrose agar, washed in sterile normal saline, and stored at 4°C until used.

Testing for antifungal activity was done by the cup-plate agar diffusion method according to Vrabilc, et al. The medium used was Chrom agar medium. The drugs tested were clotrimazole, fluconazole, itraconazole and nystatin. 1 ml of Candida albicans suspension (10^8-10^9 colony forming units per ml) was thoroughly mixed with 100 ml of sterile, melted, Sabouraud dextrose agar that was maintained at a temperature of 45 °C. 20 ml aliquots of this inoculated Sabouraud dextrose agar were distributed into sterile Petri-dish plates.

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The agar was left to set and 4 cups (10 mm in diameter) were cut in each of these plates using a sterile cork borer (No 4) and the agar discs were removed. Alternate cups were then filled with 0.1 ml of each antifungal drug solutions using an adjustable pipette. The solution was allowed to diffuse in the agar at room temperature for two hours. The plates were then incubated in the upright position at 25 °C for two days. After incubation, the diameter of the inhibition zones was measured and the mean values were recorded. Antifungal drug giving more than 19 mm inhibition zone diameter was considered as sensitive. Drugs showing 15-18 mm inhibition zones were considered as intermediate. While drugs showing less than 14 mm inhibition zones were considered as resistant.

Data was analyzed using SPSS for Windows software package, version 12. Results of each of the studied diagnostic tests were analyzed using descriptive statistics. Different diagnostic tests were compared using Mc Nemar test and the level of significance was set at p < 0.05. Descriptive statistics was used to analyze baseline characteristics of the study population. Sensitivity of each test was calculated according to the following equation:

Sensitivity = True positive / True positive + False negative; according to Akobeng⁶.

Results

104 patients were found to have typical clinical symptoms and signs of candidiasis. 60 out of these 104 patients (57.7%) were found to have hyphae on wet preparation. As exhibited in Table (I), 50 samples (48.1%) showed Gram positive budding yeast cells in Gram stain smears, while 65 samples (62.5%) showed creamy and smooth colonies of Candida species on Sabouraud agar medium. Also 72 samples (80%) showed growth on corn meal agar; and 90 samples (86.5%) showed growth in Chrom agar medium. On the other hand 72 samples (69.2%) showed positive C. albicans germ tube tests.

Table I: Detection of Candida species by conventional techniques

<table>
<thead>
<tr>
<th>Laboratory technique</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>50 (48.1%)</td>
<td>54 (51.9%)</td>
<td>104</td>
</tr>
<tr>
<td>Sabouraud agar medium</td>
<td>65 (62.5%)</td>
<td>39 (37.5%)</td>
<td>104</td>
</tr>
<tr>
<td>Chrom agar medium</td>
<td>90 (86.5%)</td>
<td>14 (13.5%)</td>
<td>104</td>
</tr>
<tr>
<td>Corn-meal agar medium</td>
<td>72 (80%)</td>
<td>15 (14.4%)</td>
<td>90</td>
</tr>
<tr>
<td>Germ tube test</td>
<td>72 (69.2%)</td>
<td>15 (14.4%)</td>
<td>90</td>
</tr>
</tbody>
</table>

On chrom agar medium, 87 samples showed green colonies suggesting C. albicans growth; one sample showed pink colonies of C. glabrata, one sample showed creamy colonies of Candida

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parapsilosis, one sample showed blue colonies of C. tropicalis, and 3 samples showed mixed colonies. Considering the significant difference and sensitivity of the laboratory techniques employed in this study, Gram stain test shall be taken as a gold standard test.

Fig. (1) shows the in vitro sensitivity testing of C. albicans against clotrimazole, fluconazole, itraconazole and nystatin. 63 isolates (70%) were found sensitive to clotrimazole, while 9 isolates (10%) were found sensitive to nystatin.

Fig. 1: In vitro sensitivity of Candida albicans against antifungal drugs

Comparing chrom agar medium and Gram stain, 42 samples were found positive by Gram stain and showed positive growth of Candida species on chrom agar medium. 6 samples were negative by Gram stain and did not show any growth on chrom agar medium. Furthermore, 48 samples showed growth on chrom agar medium but were negative by Gram stain. Also 8 samples were not able to show growth on chrom agar medium but they were positive by Gram stain; i.e. significant difference (p = 0.00); and sensitivity is 84%.

As regard Sabouraud agar, 31 samples were positive by Gram stain and showed growth on Sabouraud agar. Also 20 samples were negative by Gram scan and showed no growth on Sabouraud agar. On the other hand, 19 samples were positive by Gram stain but showed no growth on Sabouraud agar. Also 34 samples were negative by Gram stain but they were able to grow on Sabouraud agar; i.e. significant difference (p = 0.05); and sensitivity is 62%.

Comparing chrom agar and Sabouraud agar, 61 samples were able to grow on both chrom agar medium and Sabouraud agar medium; while 10 samples were not able to grow on both chrom agar

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medium and Sabouraud agar medium. Also 29 samples were able to grow on chrom agar medium but they did not grow on Sabouraud agar medium. In addition, four samples were not able to grow on chrom agar medium but they were able to grow on Sabouraud's agar medium; i.e. a significant difference was observed between these two culture media (p = 0.00); and sensitivity is 93.8%.

The women investigated were aged 15-49 years. 75 women (72.1%) were in the age range 20-29 years. Most of the women enrolled in the study (74%) were pregnant at the third trimester; while one patient (1%) was pregnant at the first trimester. 93 women (89.4%) of the study group were multigravida. The majority of the pregnant women 67 (64.4%) included in this study had primary education; while 11 women (10.6%) were university graduates. 101 pregnant women (97.1%) were housewives. 89 women (85.6%) were married to laborers and 15 (14.4%) were married to employees.

Regarding vaginal candidiasis clinical picture, 89 pregnant women (85.6%) had itching and 88 patients (84.6%) had past history of vaginitis. 104 patients had whitish discharges, 90 patients (86.5%) had copious discharge and 86 patients (82.7%) had offensive odor discharge.

Discussion

Vaginal candidiasis is a common gynecological problem, which is usually ignored in many countries including Sudan. It is frequently a distressing infection in women of child bearing age. Approximately 75% of all women had at least one episode of vaginal candidiasis during their lifetime.

This study was carried out in order to isolate and identify Candida species from vaginal discharge of pregnant women by conventional methods. It was aimed to evaluate the effectiveness of techniques employed in the diagnosis of candidiasis. The prevalence rate of vaginal candidiasis in some studies ranged from 9% to 30-40%. In this study the frequency of vaginal candidiasis among pregnant women attending Omdurman Maternity Hospital was 32.6%.

A frequency of 34% and a prevalence rate of 33.3% vaginal candidiasis among pregnant women were reported by some workers\(^4\).

Higher results had been recorded by Waboso\(^4\) who found a frequency rate of 68.2% vaginal candidiasis among pregnant women. However, lower results had been reported by Sobel\(^1\). Higher result may be attributed to selection of pregnant women complaining of vaginal discharge in the most of these studies. In this study 48% of vaginal candidiasis cases were positive by Gram stain. Another study reported the positivity of Gram stain to be 29-49%\(^1\). Also in this study chrom agar medium showed a better growth rate (86.5 %) of Candida species than Sabouraud agar medium (62.6%). Similar result was reported by Murray\(^5\) who found a better growth rate of chrom agar than that of Sabouraud agar. Candida albicans was the predominant isolated species (81%) in this study. This finding had been documented by other investigators. Also the second isolated species in this study was C. glabrata. This result was similar to that reported by other investigators\(^1\) who found that C. albicans was the most common isolated species followed by C. glabrata. Furthermore in this

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study the total frequency rate of non-\textit{C. albicans} isolates was 18.9%. This result was also similar to that given by Crislip\textsuperscript{6} who reported a non-\textit{Candida albicans} rate to be 19.3%. However it doesn’t agree with Vrabilc, \textit{et al}\textsuperscript{7} who reported a frequency rate of 8%.

In the present context, azole antifungal drugs including clotrimazole were more sensitive (70%) than nystatin (10%). This finding agrees with results of other studies\textsuperscript{8}.

In this study, most \textit{Candida} infections were in the age range 20-29 years and during the third trimester of pregnancy. A similar study done by Lissak\textsuperscript{9} showed that 78% of \textit{Candida} infections were at the same age range and in the third trimester. Also, others reported the highest attach rate of \textit{Candida} infections in the third trimester\textsuperscript{9}.

On the other hand, in this study, a high frequency rate of candidiasis was found among patients (89%) who had a low level of education. Similar result was reported by Lissak\textsuperscript{9} who found that vaginal candidiasis was more common among primary educated pregnant females.

In addition, in this study, multigravidae had a higher frequency rate (73.1%) of vaginal candidiasis than primigravidae (10.6%). This supports other workers\textsuperscript{1} who reported an increase in frequency rate of vaginal candidiasis among multigravidae. Also Omer\textsuperscript{2} reported that multigravidae suffered significantly more from vaginal candidiasis than primigravidae.

Most patients in this study (96.1%) were housewives and most of them (87.5%) were married laborers. Similar results were reported by Ortishi\textsuperscript{3} who found that the frequency rate of vaginal candidiasis was higher (94.6%) among housewives and most of them (85%) were married laborers. The common presenting signs and symptoms in this study were marked vaginal discharge (100%) and vaginal itching (70%). Others\textsuperscript{10} found that 96% of pregnant women had vaginal discharge and 84% of them were complaining of itching.

From this study, it may be recommended that all pregnant women attending antenatal care clinics should be investigated for vaginal candidiasis; and antifungal sensitivity tests should be done on a routine basis.

Conclusion: The frequency rate of vaginal candidiasis was 32.6% among pregnant women, and \textit{Candida albicans} was the commonest (81%) species isolated. The most effective antifungal drug for treatment of vaginal candidiasis was clotrimazole which was found superior to nystatin.

References


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